c.) Remarks

The applicants address each of the examiner's comments in turn.

1 and 2. Claim Status

Applicants direct the examiner's attention to their comments in section (b) above.

3. Restriction Requirement

Applicants respectfully maintain their traverse of the examiner's restriction requirement in this application, but nonetheless proceed consistently with the requirement.

4. Election of Species

Applicants affirm the election of species.

5. Further Restriction

Applicants amendments obviate the examiner's restriction in point 5 of the office action.

6. Information Disclosure Statement

Applicants acknowledge receipt and entry of the IDS filed June 23, 2000.

7. Drawings

Applicants respectfully traverse the examiners objection to the drawings. The Examiner appears to be referring to page 10 of the application which mentions structure A and structure B under a description of Figure 1 and Figure 2. In fact the sentences referring to structure A and structure B are not part of the description of the Figures, but refer to the Tables on pages 12 to 89 and 90 to 164 of the application. Therefore the drawings as presently on file are not meant to refer to structure A and structure B, and are thus correct.

8. Sequence Listing

Applicants acknowledge receipt approval of sequence listing.

9. Sequence Listing

Applicants are herein amending the specification at page 10 to refer to the SEQ ID NO for the sequence shown on page 11 of the application.

10. Rejection of Claims 12, 14, 37, 39, 40, 52, 55, 57, 58 under 35 USC § 112, second paragraph

10A: The claims as currently pending as of this response have been limited to a DAOCS or DAOCS/DACS enzyme. It is clear in the claims that the modifications are made in these enzymes. Applicants assert that the claims conform to the requirements of 35 USC § 112, second paragraph in this regard and respectfully request the examiner to withdraw this rejection accordingly.

10B: Applicants respectfully traverse this rejection. The claims refer to modifications at the site which binds the side chain of penicillin N. The application provides the structure of the DAOCS enzyme. This structure allows identification of residues in DAOCS which bind the substrate, and in particular those which bind to the side chain of penicillin N. Thus it would be clear to the skilled person whether or not any given modified DAOCS enzyme fell within the scope of the claims because they could determine from the structure in the application whether or not the position of modification corresponded to a residue which bound the penicillin N side chain. Figure 2 of the application also shows how a penicillin nucleus binds with residues of the enzyme structure. Therefore, applicants respectfully assert that the scope of the pending claims would be clear to the skilled artisan and the pending claims conform to the requirements of 35 USC § 112, second paragraph. Applicants respectfully request the examiner to withdraw this rejection.

10C: The phrase "significant sequence similarity" has been deleted from the claims and therefore this rejection has been obviated by the applicant's amendments. Accordingly, Applicants respectfully request the examiner to withdraw this rejection.

10D: Applicants respectfully traverse this rejection. The structure provided in the application shows amino acids in DAOCS which are close enough to each other to be capable of a binding interaction. Whether or not they have a binding interaction can also be determined, for example based on whether they are able to have hydrogen bond, electrostatic or hydrophobic interactions with each other. Similarly the structure can be used to deduce whether mutations of such pairs of amino acids will create or delete a binding interaction, for example by determination of whether or not the resultant mutated pair of amino acids will be capable of a binding interaction. Therefore, whether or not a mutation in DAOCS created or deleted a binding interaction would be clear to the skilled person, and therefore the scope of the claims is clear in this regard. Accordingly, Applicants respectfully request the examiner to withdraw this rejection.

11. Rejection of Claims 12, 14, 37, 39, 40, 52, 55, 57, 58 under 35 USC § 112, first paragraph (Enablement)

11(1): As discussed above the pending claims, as amended, are directed to a modified DAOCS or DAOCS/DACS enzyme. The "significant sequence similarity" language has be removed. Accordingly, Applicants respectfully request the examiner to withdraw this rejection.

11(2): Applicants respectfully traverse this rejection. As discussed above, the binding site of the side chain of penicillin N is defined in the structure provided by the application. The binding site consists of the amino acids that line the pocket in which the side chain of penicillin N lies. Thus from the structure provided in the application, it would

be clear to the skilled person which residues form the binding site, and thus the claims are enabled in this respect. Applicants respectfully request the examiner to withdraw this rejection.

11(3): Different hydrophobic amino acids differ in their level of hydrophobicity. Thus in the present case Leu158 could be modified to a more hydrophobic amino acid, such as valine or isoleucine. This aspect of the invention would therefore be clear and the guidance so provided to the skilled person would be sufficient to practice the claimed invention. Applicants respectfully request the examiner to withdraw this rejection.

11(4): As discussed above the DAOCS structure provided by the application allows the skilled person to ascertain the interactions which occur between the amino acids of DAOCS, and also allows the prediction of mutations that could create or delete a binding interaction. Therefore claims adequately enable the skilled artisan to practice the invention as claimed. Applicants respectfully request the examiner to withdraw this rejection.

12. Rejection of Claims 40, 52, 55, 57, and 58 under 35 USC § 112, first paragraph (Enablement

Applicants respectfully traverse this rejection. The Examiner argues that it is not possible to predict the effect of a change in an amino residue on the structure of DAOCS. However, applicants assert that it is this precise point which is addressed by the present invention. The invention provides the structure of DAOCS allowing prediction of the effect of modifications. Using the structure, DAOCS may be modified by "rational design" as the topology and interactions of each amino acid is now known. Thus contrary to the Examiner's assertion, the application does provide substantial guidance as to the effect of each amino acid change because the structure can be used to determine the change in binding interactions that will occur.

In particular the structure allows identification of residues in DAOCS which determine substrate specificity and further allows the skilled person to deduce the specific mutation that will be needed to change substrate specificity in a defined manner.

Thus the present invention provides modified enzymes which have defined altered substrate specificities. In particular, as discussed in the application, modified enzymes are provided which are able to utilize penicillin G. This has a hydrophobic side chain, whereas the natural substrate penicillin N has a hydrophilic side chain. Such modified enzymes which accept penicillin G as a substrate are eagerly desired in the art because the resultant product, cephalosporin G is easy to purify due to its hydrophobic side chain.

The claims relate to the use of the 3-dimensional structure of DAOCS disclosed in the present application to modify DAOCS so that it has improved activity compared to that of the wild-type enzyme. The specification provides sufficient information for the skilled person to do this. It would be a routine task for the skilled person to use the structure of DAOCS disclosed in the present application to design modified enzymes. The structure disclosed in the present application shows the substrate binding site. Mutating the residues that define the substrate binding site will alter the substrate specificity of the enzyme. Using this information the skilled person can alter substrate specificity so that a defined substrate is accepted by mutating a residue in the active site to one which allows the defined substrate to fit into the substrate binding site, i.e. since the amino acid residues defining the substrate binding site are now known they can be mutated to change the binding properties (defined by the shape and charges of the amino acids) of the substrate binding site in a desired manner.

We enclose a brief experimental write-up showing the design of a modified DAOCS enzyme that contains a leucine to valine mutation at position 158. This residue was selected because the DAOCS structure shows that it binds to the penicillin side chain. Thus mutation of this position would allow the enzyme to accept a penicillin with a different side chain,

such as penicillin G. The mutant L158V enzyme was found to have an activity towards penicillin G that was 217% that of the wild-type enzyme. In fermentation reactions using P.chrysogenum transformed with a gene encoding the L158V mutant and a wild-type gene the product yield was 570% higher from the L158V mutant strain.

In addition, we are providing herewith a number of journal articles which disclose examples of modified enzymes that have been produced using the structure of DAOCS disclosed in the present application

The DAOCS structure was used to create modified enzymes with altered cosubstrate binding. In Lee et al (European Journal of Biochemistry 270, 1301-1307, 2003) and Lee et al (Journal of Biological Chemistry 276, 18290-5, 2001), R258 was selected as a candidate for mutation since it is located in the 2-oxoglutarate binding pocket of DAOCS. Site-directed mutagenesis was used to replace this arginine residue with various different amino acids. All of the mutants produced had a broader cosubstrate selectivity than the wild-type enzyme and were able to utilize hydrophobic 2-oxoacids.

Further experiments have been carried out on C-terminally truncated versions of DAOCS and related enzymes designed using the structure of DAOCS provided herewith. The impetus to truncate the C-terminus of DAOCS came from the finding from the crystal structure of DAOCS that the C-terminus of one molecule of DAOCS is inserted into the active site of its neighbor in a cyclical fashion within a trimeric unit, which hinders the generation of a crystalline enzyme-substrate complex. Lee et al (Journal of Molecular Biology 308, 937-948, 2001) used this to design C-terminally truncated mutants of DAOCS. These mutants had altered activities towards different substrates.

Furthermore, Lloyd et al (Journal of Biological Chemistry 279, 15420-15426, 2004) carried out experiments on deacetoxycephalosporin/deacetylcephalosporin C synthase

(DAOC/DACS), a fungal enzyme closely related to DAOCS. DAOC/DACS is a bifunctional enzyme, and carries out both ring expansion of penicillin N to deacetoxycephalosporin C and hydroxylation of the latter to deacetylcephalosporin C. The DAOCS structure has been used to identify a residue which, when mutated, abolished the hydroxylation function of this enzyme resulting in the formation of a monofunctional enzyme. In addition it was found that truncation of the C terminus of DAOC/DACS to residue 310 enhanced ring expansion of penicillin G (an unnatural substrate) by 2-fold. Thus, the crystal structure of DOACS has been used to produce modifications to a related enzyme which increases its activity in accepting unnatural substrates.

Lee et al (Biochemical and Biophysical Research Communications 292, 66-70, 2002) demonstrates the utility of the DAOCS crystal structure in the design of modified enzymes with mutations in the DAOCS active site. The paper demonstrates, *inter alia*, that M180 is involved in binding 2-oxoglutarate.

The above experimental evidence confirms that the crystal structure of DAOCS can be, and indeed has been, used by the skilled person to produce many useful mutants of DAOCS and even of related enzymes, which have increased yield of product compared to that of the wild-type enzyme or accept different substrates than the wild-type, in particular to accept penicillin G. It is thus submitted that the present application enables the skilled person to design and modify DAOCS enzymes as is currently claimed, and does not require the skilled person to carry out an undue amount of experimentation when carrying out the invention. . Applicants respectfully request the examiner to withdraw this rejection.

13. Rejection of Claims 12, 14, 37, 39, 40, 52, 55, 57, 58 under 35 USC § 112, first paragraph (Written Description)

The Examiner argues that the specification does not reasonably convey to one skilled in the art, that the inventors had possession of the invention at the time the invention was filed. The Examiner also argues that it is unclear which changes in structure are within the scope of the claims, and further that representative examples are required which provide reasonable reassurance that compounds within the scope of the claim possess the alleged utility.

At the outset, applicants note that the pending claims have been amended and do not contain the "significant sequence similarity" language. As discussed above, the amino acids which define the binding site of the side chain of penicillin N can be determined from the structure which is provided by the application. It would be a routine matter for the skilled person to identify these amino acids, and thus the structure provided in the application provides an adequate written description of this aspect of the invention. Thus, applicants assert that the specification does describe what changes in enzyme structure are within the scope of the claims, and the specification adequately teaches these to the skilled artisan.

Further, as discussed above in response to the enablement rejection, it has now been shown that modifications to residues identified using the present structure resulted in modified DAOCS enzymes with the desired properties. Therefore the Examiner's assertion that modification to amino acids in DAOCS would have an unpredictable effect is not correct. The effect is predictable as shown by the enclosed data and journal articles.

The data and articles also provide evidence that representative examples of modifications covered by the claims have the desired utility. Therefore the invention is

adequately disclosed in the application. Applicants respectfully request the examiner to withdraw this rejection.

14. Rejection of Claims 12, 14, 37, 39, 40, 52, 55, 57, 58 under 35 USC § 102(b)

The Examiner has rejected the claims under 35 USC § 102(b) citing database access accession numbers S40253, A39204 and S54101 of Database Pir 76 to argue that enzymes of the invention lack novelty. However the present claims are directed to modified enzymes, and therefore do not cover the naturally occurring enzymes mentioned by the Examiner. Therefore the present claims are novel over the cited database references. Accordingly, Applicants respectfully request the examiner to withdraw the rejection under 35 USC § 102(b).

d.) Conclusions

In light of the amendments and arguments made herein, Applicants assert that the pending claims are in condition for allowance and earnestly request same.

Applicants include a payment of $420.00 with this filing for a 2 month extension of time. It is believed that these are the only fees due with the filing of this response. However, if Applicants are in error, the Commissioner is hereby authorized to draw any additional fees associated with this filing from Deposit Account No. 06-2375, under Order No. P02005US0/10020482, from which the undersigned is authorized to draw.

Respectfully submitted,

Date: July 23, 2004

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I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail No . ER509324957US, in an envelope addressed to Mail Stop Non-Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450, on the date shown below.

Date: July 23, 2004 By Pamela Tincha (Pamela Tincha)